#8 AP 9/27/01

CERTIFICATE OF MAILING

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST-CLASS MAIL IN AN ENVELOPE ADDRESSED TO: ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231, ON SEPTEMBER 19, 2001

Marianne H Michel
AGENT/ATTORNEY FOR APPLICANT

September 19, 2001
DATE

Attorney Docket No. 1115

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Gordon-Kamm et al.	Date:	September 19, 2001
Serial No.:	09/511,445	Group Art Unit:	1638
Filed:	February 22, 2000	Examiner:	C. Collins

For: "Methods of Using Viral Replicase Polynucleotides and Polypeptides"

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF WILLIAM J. GORDON-KAMM UNDER 37 C.F.R. 1.132

I, William J. Gordon-Kamm, declare:

I am a citizen of the United States of America and a resident of Urbandale, Iowa.

I received a degree of Doctor of Philosophy in Plant Physiology, with minors in Plant Biochemistry and Plant Cell Biology from Cornell University, Ithaca, New York, January 1985.

I received a degree of Master of Science in Botany from Western Washington University, Bellingham, Washington, June 1980.

I received a degree of Bachelor of Science in Biology from Western Washington University, Bellingham, Washington, January 1979.

I presently hold the position of Research Manager, Quality Traits Transformation Research at Pioneer Hi-Bred International, Inc., Johnston, Iowa, June 1996 to present.

I was Coordinator, Maize Elite Transformation, Pioneer Hi-Bred International, Inc., Johnston, Iowa from November 1994 to June 1996.

I was Senior Research Scientist at DeKalb Plant Genetics, Mystic, Connecticut, February 1991 to October 1994.

I was Research Scientist at DeKalb Plant Genetics, Mystic, Connecticut, from May 1987 to February 1991.

I was Assistant Professor at New Mexico Highlands University, Division of Science and Mathematics, Las Vegas, New Mexico from August 1985 to May 1987.

I was Visiting Scientist at USDA/ARS, Cereal Rust Laboratory, University of Minnesota, St. Paul, Minnesota from May 1986 to August 1986.

I was Preferred Post-Doctoral Associate at USDA/ARS, Cereal Rust Laboratory, Univ. of Minnesota, St. Paul, Minnesota from October 1985 to July 1985.

Issued Patents:

US5550318. Methods and composition for the production of stably transformed, fertile monocot plants and cells thereof. DeKalb Genetics Corp. (see also; EP0485506 & WO9102071).

US5489520. Process of producing fertile transgenic Zea mays plants and progeny comprising a gene encoding phosphinothricin acetyl transferase. DeKalb Genetics Corp.

US5874265. Methods and composition for the production of stably transformed fertile monocot plants and cells thereof. DeKalb Genetics Corp. (see also; EP0721509 & WO95506128).

US5736369. Method for producing transgenic cereal plants (meristem transformation). Pioneer Hi-Bred International, Inc. (see also; EP0772687 & WO 9604392).

US5780709. Transgenic maize with increased mannitol content. DeKalb Genetics Corp. (see also; EP0889967 & WO 9726365).

US6262341. Method for the integration of foreign DNA into eukaryotic genomes. Pioneer Hi-Bred International, Inc. (see also; EP0889967 & WO 9824608)

US6281411. Transgenic monocot plants with increased glycine-betaine content. DeKalb Genetics Corp.

US6284947. Methods of using viral replicase polynucleotides and polypeptides. Pioneer Hi-Bred International, Inc.

Journal Articles:

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GORDON-KAMM, W.J., SPENCER, T.M., MANGANO, M.L., ADAMS, T.R., DAINES, R.J, START, W.G., O'BRIEN, J.V., CHAMBERS, S.A., ADAMS, W.R., WILLETTS, N.G., RICE, T.R., MACKEY, C.J., KRUEGER, R.W., KAUSCH, A.P. and P.G. LEMAUX. 1991. Transformation of maize cells and regeneration of fertile transgenic plants. The Plant Cell 2:603-618.

SPENCER, T.M., GORDON-KAMM, W.J., DAINES, R.J., START, W.G. and P.G. LEMAUX. 1990. Bialaphos selection of stable transformants from maize cell culture. Theor. Appl. Genet. 79:625-631.

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Chapters in Books:

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meristem: Transgenic sector reorganization and germline transmission. IN: Genetics, Biotechnology and Breeding of Maize and Sorghum. (A.S Tsaftaris, ed.), Royal Soc. chemistry, Cambridge, UK, pp. 94-97.

MACKEY, C.J., SPENCER, T.M., ADAMS, T.R., LEMAUX, P.G., KAUSCH, A.P., KREUGER, R.W. and W.J. GORDON-KAMM. 1993. Transgenic maize. IN: Transgenic Plants, Vol. 2, Present Status and Social and Economic Impacts, (S-D. Kung and R. Wu, eds.), Acad. Press, Inc., San Diego, pp. 21-31.

STEPONKUS, P.L.,DOWGERT, M.F.,EVANS, R.Y. and W.J GORDON-KAMM. 1982. Cryobiology of isolated protoplasts. IN: Plant Cold Hardiness and Freezing Stress, Vol. 2, (P.H. Li and A. Sakai, eds.), Academic Press, New York, pp. 459-474.

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Selected Abstracts:

AVES, K., GENOVESI, D., WILLETTS, N., ZACHWIEJA, S., ADAMS, T., MANN, M. and W.J. GORDON-KAMM. 1992. Transformation of an elite maize inbred through microprojectile bombardment of regenerable embryogenic callus. In Vitro Cell Dev. Biol. 28 (3): 54A.

GORDON-KAMM, W.J. 1991. Factors influencing the transformation of maize by microprojectile bombardment. First Int. Symposium on Particle Mediated Transformation. Madison, WI. Mar. 13-15.

GORDON-KAMM, W.J., SPENCER, T.M., MANGANO, M.L., ADAMS, T.R., DAINES, R.J., O'BRIEN, J.V., START, W.G., ADAMS, W.R., CHAMBERS, S.A., WILLETTS, N.G., KRUEGER, R.W., KAUSCH, A.P.MACKEY, C.J. and P.G. LEMAUX. 1990. Bialaphos resistant maize transformants using microprojectile bombardment. Abstracts VIIth International Congress on Plant Tissue and Cell Culture, p. 59.

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LEMAUX, P.G., SPENCER, T.M., MANGANO, M.L., ADAMS, T.R., DAINES, R.J., O'BRIEN, J.V., START, W.G., ADAMS, W.R., CHAMBERS, S.A., WILLETTS, N.G., KRUEGER, R.W., KAUSCH, A.P.MACKEY, C.J. and W.J. GORDON-KAMM. 1990. Transformation of maize suspension cultures using the herbicide bialaphos. J. Cellular Biochem. Suppl. (Keystone Symposium).

GORDON-KAMM, W.J., ADAMS, T.R., ADAMS, W.R., CHAMBERS, S.A., COURREGES, V.C., DAINES, R.J., MANGANO, M.L., O'BRIEN, J.V., SPENCER, T.M., START, W.G., WILLETTS, N.G., KAUSCH, A.P., KRUEGER, R.W., LEMAUX, P.G. and C.J. MACKEY. 1989. Stable transformation of embryogenic maize cultures by microprojectile bombardment. J. Cell. Biochem. Suppl. (Keystone Symposium).

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maize suspension cultures using the herbicide bialaphos. FASEB conference, Plant Gene Expression, Copper Mountain, CO.

MANGANO, M.L., WILLETTS, N.G. and W.J. GORDON-KAMM. 1989. Long-term cold storage of regenerable maize callus. In Vitro Cell. Dev. Biol. 25 (3): 47A.

GORDON-KAMM, W.J. and W.R. BUSHNELL. 1986. Intracellular microinjection of protoplasts viewed by DIC optics of Hoechst Stain. Abstracts VI international Congress of Plant Tissue and Cell Culture. p. 205.

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I am personally familiar with the experiments and the method of collection of the data provided below and feel qualified to form conclusions based on the data.

Transcription and translation activity increase proportionally with each doubling of the genome, therefore the metabolic activity of a highly polyploidy cell is functionally equivalent to multiple diploid cells (D'Amato, 1984). Under natural conditions endoreduplication in plants is modulated by the levels of hormones and by light (Meyers et al., 1990; Artlip et al., 1995; Gendreau et al., 1997).

Endoreduplication is correlated with metabolic rate, terminal differentiation, gene transcription and the degree of grain filling (D'Amato, 1984; Kowles and Phillips, 1985; Brunori et al., 1993). There is also documentation of a correlation with organ growth in *Arabidopsis* hypocotyls and a correlation with degree of grain fill in maize (Gendreau et al., 1997; Kowles and Phillips,1985, 1988). In Atriplex, increasing ploidy levels are tightly correlated with photosynthetic rates and cell size in the leaf (Warner and Edwards, 1989).

Increased cell size, metabolic activity such as photosynthesis, grain fill, etc have been documented to participate in overall increases in yield, whether vegetative or in fruit or seed. Direct documentation has been reported in banana. In plantain-banana hybrids, bunch weight, fruit weight, and size was positively correlated with increased ploidy levels (Ortiz R and Vuylsteke D, 1995). Simple side-by-side comparisons of naturally occurring Masa germplasm triploid bananas have larger fruits than diploid bananas (Simmonds NW, 1976. Bananas. In: Evolution of Crop Plants, Longman Press, London & New York).

Exhibit 1 represents flow cytometric analysis of DNA content measured in soybean nuclei isolated from RepA- non-transformed cells (upper panel) and RepA+ transformed cells (lower panel). Nuclei isolated from leaf cells from non-transformed soybean plants (or from soybean containing transgenes other than RepA) exhibit two main peaks; the taller peak on the left shows the proportion of leaf cells in the G1 phase of the cell cycle and the shorter right-hand peak represents cells in G2 phase. This is a typical cell cycle profile for diploid cells.

In the lower panel of Exhibit 1, nuclei from a RepA+ transgenic soybean plant are shown in which the G1 and G2 peaks have shifted to the right, indicating an exact doubling in overall cellular DNA content (i.e. from diploid to tetraploid).

In Exhibit 2 segregating T1 progeny are shown. As indicated by the labels, the RepA+ transgenic T1 plants on the right showed a substantial increase in overall plant vigor and size, relative to transgenic T1 plants that did not contain RepA or to the wild-type parental genotype in the background.

Example 2 and Figure 1 in the above-identified application demonstrate an increase level of tetraploid cells in maize.

Therefore, we have demonstrated in soybean and in maize that after transformation of diploid cells with a RepA expression cassette, transgenic plants can be regenerated in which the ploidy level has increased from diploid to tetraploid. This means that endoreduplication occurred.

In both maize and soybean, the regenerated tetraploid plants were substantially larger than corresponding diploid plants that were not transformed with RepA. In soybean, in which successful crosses were completed with these RepA+ tetraploid plants, the resultant T1 progeny inherited this "giant" phenotype as shown in Exhibit 2. Consistent with numerous supporting examples in the scientific literature, it is my opinion that in addition to its increased vegetative stature, tetraploid lines will exhibit increased photosynthetic rates and increased yield.

Gemniviruses, similar to many mammalian viruses, must recruit host cell cycle machinery for viral DNA replication to occur. The geminivirus replicase genes, for example the wheat Dwarf virus RepA gene, are thus analogous to mammalian viral proteins such as the SV40 large-T antigen, the adenovirus E1A or the human papilloma virus E7 protein the primary instigators of this process. These proteins share a common feature, the retinoblastoma-binding domain (LxCxE), and all have been demonstrated to bind Rb. Once Rb is sequestered away from the G1-S transcription factor E2F, which is bound to one of the above proteins, progression through S-phase and the remainder of the cell cycle is stimulated (see Xie et al., 1995 for review). Proliferation of mammalian cells can be stimulated by expression of SV40 T-antigen (Moran, 1988), E1A (Iuliano et al., 2000), or E7 (Caldeira et al., 2000). It should be emphasized that in such examples this is a stimulation of the mitotic cell cycle and not endoreduplication. Because WDV RepA protein has a mode of action that is almost identical to these mammalian proteins, it would be expected that RepA expression would result in plant mitotic cell cycle stimulation. Thus, based on the biological evidence available, the utility that we claim in this application (RepA expression resulting in endoreduplication and increased ploidy levels) is not anticipated or suggested by the literature. In fact, the literature teaches against the invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true;

and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

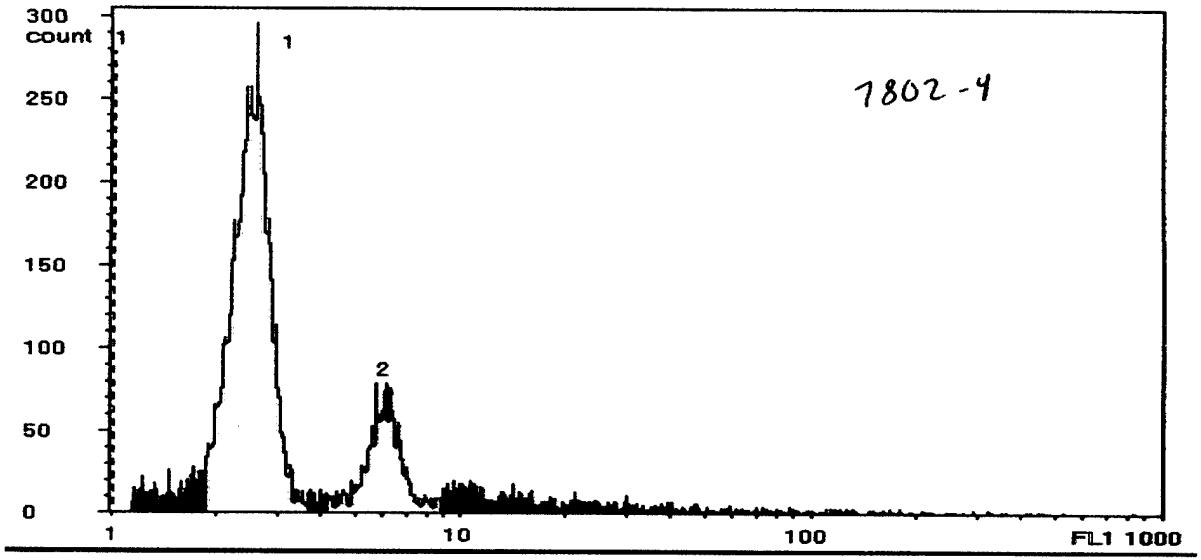
Wm Gordon-Kamm

William J. Gordon-Kamm

Sept. 19, 2001

Date

- **Jack: Old, 1/1**
  Young, 1/1
  T1 of: RepA$^-$, 5/5
  RepA$^+$, 8/8



- **T1 of 7801**
  RepA$^+$, 12/12

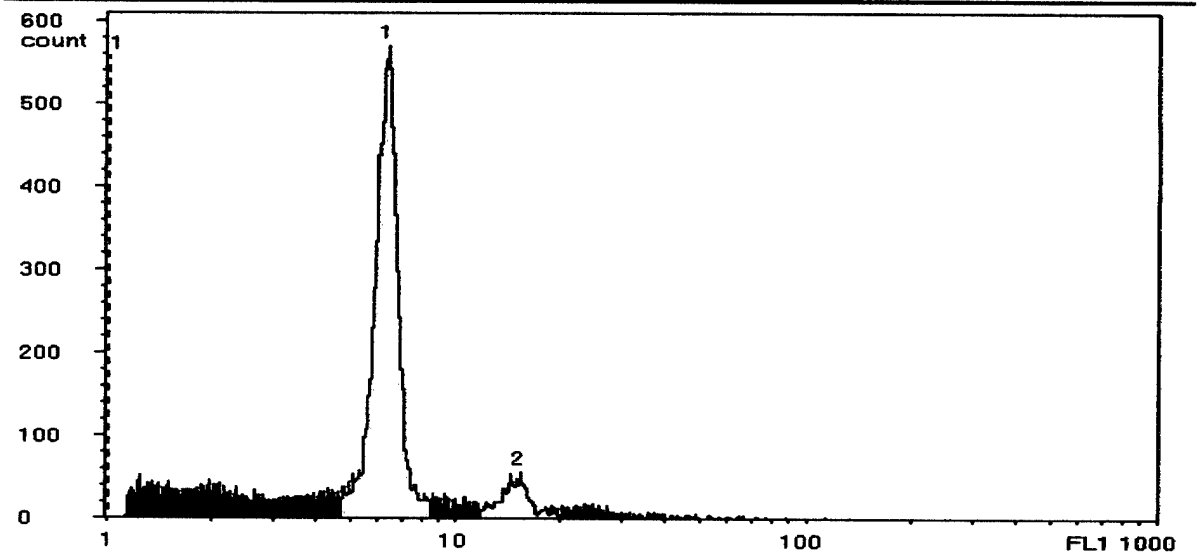


Exhibit 1

Exhibit 2